REMARKS

Specification

The Examiner objected to the disclosure on account of inconsistencies in numbering of SEQ IDs between the sequence listing and the specification. Applicant notes that this inconsistency was previously corrected by the Preliminary Amendment dated July 30, 2004 that was entered into the record on August 5, 2004. Thus no additional corrections should be necessary at this time.

Cancelled and Withdrawn Claims

Claims 39-72 and 94-214 have been cancelled solely on the basis that they are directed to non-elected inventions. The withdrawn claims that remain pending are drawn to the elected invention, but to a non-elected species thereof. Upon allowance of the claims drawn to the elected species, consideration of these claims drawn to a non-elected species is respectfully requested.

Claim Objections

Applicant has amended Claims 6, 80, and 81 to correct the informalities noted by the Examiner in paragraphs 3-5 of the Office Action.

Claim Rejections under 35 U.S.C. §112

Applicant has amended Claims 1, 4, 27, 29, 34, 35, 73, and 78 to address concerns regarding indefiniteness noted by the Examiner in paragraphs 8-14 of the Office Action.

In response to the Examiner's rejection in paragraph 8 of the Office Action, Applicant has amended Claim 1 to clarify that the lysate comprising mRNA includes the specific mRNA.

In response to the Examiner's rejection in paragraph 9 of the Office Action, Applicant has amended Claims 4 and 78 to clarify that the whole blood is thawed prior to filtration.

In response to the Examiner's rejection in paragraph 10 of the Office Action, Applicant has amended Claim 27 to clarify that the mRNA to be quantified is mRNA known to be induced during apoptosis development in leukemia. One of ordinary skill in the art can easily identify mRNA known to be induced during apoptosis development in leukemia. One example of such

mRNA is mRNA that encodes p21, which is disclosed, for example, in Figure 22C and Paragraph [0134] of the specification.

In response to the Examiner's rejection in paragraph 11 of the Office Action, Applicant has amended Claim 29 to clarify that the quantification of mRNA is used to test the side effects of anti-cancer drugs that induce specific mRNA responsible for apoptosis development in leukocytes.

In response to the Examiner's rejection in paragraph 12 of the Office Action, Applicant has amended Claim 34 to clarify that the whole blood is exposed to donor cells prior to filtration.

In response to the Examiner's rejection in paragraph 13 of the Office Action, Applicant has amended Claim 35 to explain how quantification of mRNA of donor cell-mediated cytokines is used to test transplant rejection, clarifying that a higher than normal level of mRNA of donor cell-mediated cytokines is indicative of transplant rejection. One of ordinary skill in the art will readily recognize that a higher than normal level of mRNA of donor cell-mediated cytokines is indicative of transplant rejection.

In response to the Examiner's rejection in paragraph 14 of the Office Action, Applicant has amended Claim 73 to clarify how the values in steps (g) and (h) are calculated.

These amendments should thereby overcome the rejections under 35 U.S.C. § 112 with respect to these claims and all claims dependent thereupon.

Claim Rejections under 35 U.S.C. §103

Claims 1, 3, 5, 8, 11, 12, 15, and 36-38

The Examiner rejected Claims 1, 3, 5, 8, 11, 12, 15, and 36-38 as unpatentable over Ishikawa *et al.* in view of Mitsuhashi and Garvin. Applicant has amended Claim 1 to clarify that the leukocytes obtained after filtration of whole blood comprise eosinophils. Support for this amendment can be found in Paragraph [0094] of the specification.

Applicant's use of filtration to obtain leukocytes results in collection of most or all subsets of leukocytes, including eosinophils. Eosinophils contain a large quantity of RNases. By contrast, the centrifugation method employed by Ishikawa *et al.* uses a density gradient to collect mononuclear leukocytes, in which the RNase content is very small. One skilled in the art would not have any reason to retain the RNase-rich leukocytes when conducting an assay for mRNA, as the RNases present in leukocytes would be expected to degrade the mRNA being assayed. As a

result, it would be counter-intuitive for one skilled in the art to combine the teachings of Ishikawa *et al.* with those of Mitsuhashi and/or Garvin. However, applicants have unexpectedly discovered that the degradation of the mRNA can be avoided by lysing the leukocytes with an appropriate lysis buffer directly on the filter membrane to produce a lysate that contains the mRNA. Because it is only Applicant's discovery that would lead to a combination of the references cited by the Examiner, the cited references do not create a *prima facie* showing of obviousness.

Moreover, even if one having ordinary skill in the art were to combine Ishikawa *et al.* with Mitsuhashi and Garvin in the manner suggested by the Examiner, none of these references would lead such a person to predict the significant unexpected results obtained by the combination. The Examples in the specification directly compare the results obtained using the claimed method with that of conventional density gradient separation of peripheral blood mononuclear cells (PBMC) as described in Ishikawa *et al.* For example, in Example 7, the disclosed filtration method results in significantly improved recovery of mRNA compared to conventional density gradient and centrifugation separation methods like that used by Ishikawa *et al.* *See* Spec., Paragraph [0134]. The disclosed filtration method also results in superior uniformity of mRNA recovery. *See* Spec., Paragraph [0135]. In addition, a wide range of lysis buffer concentrations are suitable for optimum performance, demonstrating reproducibility and robustness. *See* Spec., Paragraph [0137].

Furthermore, as shown in both Examples 5 and 7, the total assay efficiency is sequence-independent. *See* Spec., Paragraphs [0125] and [0143]. On account of these significant unexpected results and in light of the long-felt need in the field for a high-throughput method of quantifying mRNA, Ishikawa *et al.* taken in view of Mitsuhashi and Garvin does not render Claim 1 obvious. These unexpected results are strong evidence of nonobviousness that would rebut a *prima facie* showing of obviousness even were such a showing present.

The foregoing amendments should thereby render Claim 1 and all claims dependent thereupon in condition for allowance. Moreover, as discussed below, many of the dependent claims recite limitations that provide additional reasons for their patentability.

Claim 36

Although the foregoing amendments to Claim 1 should automatically render Claim 36 in condition for allowance based upon its dependence upon Claim 1, in light of the Examiner's remarks Applicant wishes to provide additional reasons why Claim 36 is nonobvious. Claim 36 recites the additional limitation of determining the quantity of target mRNA in the sample using spiked control RNA. The recovery of control RNA should be the same for various target mRNAs for the control RNA in question to serve as a universally applicable control RNA. However, the recovery of control RNA may vary depending on the length, sequence, and abundance of mRNA. Thus, prior to Applicant's invention, no consensus was previously available whether development of a universal control RNA was even possible. Thus, Claim 36, which recites the use of such spiked control RNA, is nonobvious in view of the disclosure of the cited references for this additional reason as well.

Claim 37

Additionally, Claim 37 recites that specific antisense primers are applied during the lysate transferring step. The importance of this limitation is discussed in Paragraph [0080] of the specification; the addition of these primers serves as an important distinction from prior art techniques. The cited Ishikawa *et al.* reference discloses the use of an oligo(dT) primer which is immobilized on a solid support. Any mRNA that is bound to the oligo(dT) can be extended using the oligo(dT) as a primer. Thus all of the primed cDNA that is generated in Ishikawa *et al.* is immobilized on a solid support until it is denatured.

By contrast, through the addition of specific antisense primers, Applicant achieves release of cDNA into the solution phase without the necessity of heat denaturation. As described in Paragraph [0080] and shown in Figure 15, cDNA is primed by both the oligo(dT) and by the antisense primers. The cDNA derived from extension of the antisense primers is displaced by cDNA derived from extension of the bound oligo(dT). Thus, the cDNA derived from extension of the antisense primers can enter the solution phase following displacement by the oligo(dT)-derived cDNA during amplification. Remarkably, this cDNA displacement occurs without heat denaturation. Nothing in the Ishikawa *et al.* reference, or any of the other references cited by the Examiner, discloses this feature. Accordingly, no *prima facie* showing of obviousness of Claim 37 can be set forth by the cited combination of references for this additional reason as well.

Claims 6 and 7

The Examiner rejected Claims 6 and 7 as unpatentable over Ishikawa *et al.* in view of Mitsuhashi and Garvin and in further view of Pall. The foregoing amendments to Claim 1 should automatically render Claims 6 and 7 in condition for allowance based upon their dependence upon Claim 1.

Claims 8 and 9

The Examiner rejected Claims 8 and 9 as unpatentable over Ishikawa *et al.* in view of Mitsuhashi and Garvin and in further view of Naef. The foregoing amendments to Claim 1 should automatically render Claims 8 and 9 in condition for allowance based upon their dependence upon Claim 1. However, in light of the Examiner's remarks Applicant wishes to provide additional reasons why Claim 8 is nonobvious. Leukocyte preparation using a hypotonic solution wash coupled with a centrifugation method of collecting leukocytes reduces the recovery of mRNA. This may be because some leukocytes are damaged, resulting in mRNA leakage during the centrifugation step. However, employing a hypotonic solution wash with a filtration method of collecting leukocytes does not result in an analogous reduction in mRNA recovery. Thus, even though washing with a hypotonic solution was known, employing a hypotonic solution wash on a filter is unique and nonobvious.

New Claims

Claims 215-219 have been added. Support in the specification for these claims can be found in Paragraph [0080] of the specification as filed. No new matter is added thereby.

These new claims are patentable over the references cited in the Office Action. These claims recite a method that includes a step similar to that recited in Claim 37 in which a lysis buffer is used that contains antisense primers specific to the specific mRNA. As discussed above in connection with Claim 37, the addition of such primers allows release of cDNA derived from extension of the antisense primers to enter the solution phase following displacement by oligo(dT)-derived cDNA during amplification even without heat denaturation. The provision of such cDNA in solution is nowhere disclosed by the cited references. Accordingly, the new claims are presented as nonobvious in view of Ishikawa *et al.*, even in view of any of the additional references cited by the Examiner.

Double Patenting

The Examiner provisionally rejected Claims 1, 3, 4, and 8 on the ground of nonstatutory obviousness-type double patenting over Claims 1-5 of copending Application No. 11/376,018. Applicant requests deferral of this provisional rejection until such time as the conflicting claims are allowed in one of the two applications.

No Disclaimers or Disavowals

Although the present communication may include alterations to the application or claims, or characterizations of claim scope or referenced art, the Applicant is not conceding in this application that previously pending claims are not patentable over the cited references. Rather, any alterations or characterizations are being made to facilitate expeditious prosecution of this application. The Applicant reserves the right to pursue at a later date any previously pending or other broader or narrower claims that capture any subject matter supported by the present disclosure, including subject matter found to be specifically disclaimed herein or by any prior prosecution. Accordingly, reviewers of this or any parent, child or related prosecution history shall not reasonably infer that the Applicant has made any disclaimers or disavowals of any subject matter supported by the present application.

Co-Pending Applications of Assignee

Applicant wishes to draw the Examiner's attention to the following co-pending applications of the present application's assignee.

Serial Number	Title	Filed
11/803,663	DEVICE AND METHOD FOR HIGH-THROUGHPUT QUANTIFICATION OF MRNA FROM WHOLE BLOOD	May 15, 2007
11/803,594	DEVICE AND METHOD FOR HIGH-THROUGHPUT QUANTIFICATION OF MRNA FROM WHOLE BLOOD	May 15, 2007
11/803,593	DEVICE AND METHOD FOR HIGH-THROUGHPUT QUANTIFICATION OF MRNA FROM WHOLE BLOOD	May 15, 2007
11/525,515	DEVICE AND METHOD FOR HIGH-THROUGHPUT QUANTIFICATION OF MRNA FROM WHOLE BLOOD	September 22, 2006

11/376,018	DEVICE AND METHOD FOR HIGH-THROUGHPUT QUANTIFICATION OF MRNA FROM WHOLE BLOOD	March 15, 2006

The Applicant understands that the Examiner has access to sophisticated databases available within the USPTO that will allow full access to the file histories of these applications. As such, Applicant respectfully requests that the Examiner review these file histories for any actions that may be relevant to the prosecution of the present application.

CONCLUSION

In view of the foregoing, the present application is believed to be fully in condition for allowance. However, should any remaining impediments to allowance be identified by the Examiner, the Examiner is respectfully invited to contact the undersigned attorney at the telephone number appearing below.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

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Dated: 17 Dec. 2007

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